GIANT AXONS AND SYNERGIC CONTRACTIONS
IN BRANCHIOMMA VESICULOSUM

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(Received 2 June 1950)

(With Eight Text-figures)

INTRODUCTION

Conduction in the giant axons of annelids has been clarified by the work of Stough, Eccles and colleagues, Bullock, Rushton and others (reviewed by Nicol, 1948 a). They are now known to be motor axons, or at least nerve fibres on the efferent side of reflex arcs, and they intermediate quick withdrawal movements effected by synergic contractions of widespread longitudinal muscles. Probably the most highly developed axons of this kind occur in the Serpulimorpha (Nicol, 1948 a, b, c, d). Our knowledge of the physiological activity of these nerve fibres, as components of a neuro-muscular unit, is still incomplete, however, and the sabellid worm Branchiomma vesiculosum (Montagu) has been investigated, as a suitable experimental animal. The present paper gives an analysis of giant axon functioning in the intact animal as revealed through muscular activity. A few observations on the functioning of the giant fibres in Myxicola infundibulum (a related species) have already been published (Nicol, 1948 c), and will be referred to in the present account. Budington (1902) described the results of stimulating pieces of the earthworm which contained nerve cord. His records show contractions of the longitudinal muscles, and it is probable that he was stimulating the giant axons of the animal.

An account of the histological organization of the giant axons of Branchiomma is the subject of a complementary paper. This is summarized as follows. There are two giant axons in this species (Fig. 1), and each axon arises from a large cell in the posterior part of the supraoesophageal ganglia. The two fibres decussate in these ganglia, and each then descends through the circumoesophageal connective to reach the ventral nerve cord. A giant axon lies in each half of the nerve cord and runs to the posterior end of the abdomen. The giant axons are certainly syncytial structures connected with a number of nerve cells, but no attempt has been made to discover all their neurocytons.

An important feature of the organization of the giant axons of Branchiomma lies in the arrangement and distribution of the motor branches to the muscles. In each segment a giant axon gives off lateral branches which proceed directly to the ipsilateral longitudinal muscles of that segment (Fig. 1). These motor branches run on the internal faces of the muscle masses, and give off finer twigs which descend into the muscle mass among the muscle fibres. The giant axons in the supraoesophageal ganglia are about 10 μ in diameter; in the thoracic nerve cord they increase to 230 μ,
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and gradually taper off posteriorly, to reach fine dimensions of about 7 μ or less in the tail. The motor branches are about 15 μ in diameter at their origins in the nerve cord, and they dwindle to fine filaments among the muscles.

The structure of polychaete muscles has been reviewed by Prenant (1929). Apparently the longitudinal muscles of *Branchiomma* and other sabellids are nematoid in form and consist of longitudinal ribbons which are attached to connective tissue membranes, and are invested by connective tissue fibres. Each ribbon contains a flattened contractile plate, folded on itself to form a compressed U. The plate is the contractile element and contains myofibrils, but lacks striations. Sarcoplasm bulges from an orifice in the plate, surrounds the fibre as a sheath, and contains a nucleus; along one edge of the fibre the sarcoplasm may form a peculiar frilled membrane. Circular and parapodial muscles appear to be similar. Consequently, these muscles seem to be rather unusual types of smooth muscle (Brunotte, 1888; Hanson, 1948; Jourdan, 1887; Prenant, 1929). Both Brunotte and Prenant regarded the longitudinal muscles as syncytial structures in which the contractile plates lie in an anastomosing sarcoplasm.

METHODS

Specimens of *B. vesiculosum* used in this investigation were kept under circulation in the laboratory. For an investigation of the functioning of the giant fibres and longitudinal muscles the entire animal forms a convenient neuro-muscular preparation. Animals were removed from their tubes and studied in sea water. When the animal is stimulated mechanically by touching it with a glass rod, it shortens abruptly. This reaction involves a complete reflex arc beginning with epidermal receptors which fire the giant axons in the nerve cord, the giant axons forming the efferent side of the arc. Electrical stimuli delivered to the surface of the animal stimulate the giant axons directly, resulting in synergic contraction of the longitudinal muscles and shortening of the animal.

For recording contractions both isotonic and isometric levers were used. A hook was passed transversely through the anterior end of the animal and connected to the
recording lever. The anterior end (head and thorax) is rather firm and hard, owing to dense musculature and a turgid endoskeleton supporting the branchial crown. The posterior end was pinned down on a cork surface. Isotonic levers were lightly weighted to return the animal to its original length and to cause the writing point to return to base-line. Certain characteristics of the preparation that present difficulties in recording will be referred to in the observations. Here it is noted that the animal is very extensible. Under stimulation and recording it gradually fatigues and elongates, and it is necessary to keep adjusting the isotonic lever to maintain some light tension and to take up the slack. Again the tissues of the animal, particularly those of the posterior abdomen, are soft and easily torn; this restricts considerably the tension to which the animal can be subjected in making a taut line to an isometric lever. Despite these limitations favourable preparations give clear-cut results.

Fig. 1. Isotonic contractions following stimulation of the giant axons of Branchiomma vesiculorum. Upper line, contractions of the animal; lower line, time scale at 1 per sec. Stimulation frequencies: a, 12 per min.; b, 36 per min.; c, 62 per min.; d, 124 per min.; e, 164 per min.; f, 13 per sec.

Electrical stimuli were applied through a pair of platinum electrodes resting on the dorsal surface of the animal. Condenser discharges from a 0.1 μF condenser were used. Frequency of stimulation was controlled by a hand key, metronome with mercury cups, or a commutator, in the discharge circuit. A minimal effective voltage was used, and this varied with the preparation (6-18 V.; usually 7.5 V. were effective).

**OBSERVATIONS**

*Stimulation of the giant axons*

Single discharges at intervals of 5 sec. to 2 sec. from a 0.1 μF condenser, at constant voltage, produce single muscle twitches (Fig. 2a, b). At higher frequencies (1-2 stimuli per sec.) relaxation is incomplete between individual contractions and clonus results (Fig. 2c, d). At still higher frequencies (about 3 stimuli per sec.) typical tetanus ensues (Fig. 2e, f). These figures have relative significance only, since tetanus appears at lower frequencies of stimulation with onset of fatigue (Fig. 3). In the earthworm, Budington (1902) found that, with isotonic recording
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of longitudinal muscular contractions, tetanus developed at a stimulation frequency of 4 per sec.

It is characteristic of this animal that it is unable to maintain sustained contractions for long periods and progressive fatigue sets in, particularly at high rates of stimulation. The fatiguing process is reversible; after brief periods of rest the animal recovers the ability to contract during giant axon stimulation, but to a lesser degree than before. Longer periods of rest effect greater degree of recovery (Fig. 4).

Isotonic twitches frequently show an interesting delayed effect in that the lever does not immediately return to base-line, but the descent is delayed by secondary prolonged contractions (Fig. 2a, b, c, d). The tension developed by these secondary contractions is rather low, since they do not show in isometric records. They are
apparently the result of enhanced tonic contractions in the longitudinal muscles following giant axon stimulation. An analogous process occurs in *Myxicola infundibulum* in that quick isotonic twitches are followed by periods of slow elongation (Nicol, 1948c).

**Time course of contractions**

Little information is available concerning the time course of contractions in annelid somatic muscle. Data are here presented for contractions in the longitudinal musculature following giant axon stimulation. Single twitches were recorded with a semi-isometric lever (on rather resistant steel-wire). Stimuli, by condenser discharges, were delivered at various rates; in Fig. 5 stimuli were 82 and 164 per min. consecutively, and the records show the progress of fatigue.

![Fig. 5. Isometric contractions. Time scale, 1 per sec. Stimulation frequencies: a, 82 per min.; b, 164 per min. Effects of fatigue shown in extension of relaxation time, and reduction in tension developed.](image)

Single contractions or twitches resulting from giant axon stimulation are fairly rapid. Maximal tension in this experiment (stimulation rate 82 per min.) developed 255 msec. after the beginning of contraction. Relaxation required about 1.8 sec. for completion. After 10 stimuli at 82 per min. the time course of contraction was considerably prolonged, maximal tension being attained after 475 msec., and relaxation being completed after about 3 sec. In *M. infundibulum* (another sabellid worm), single isotonic twitches were found to occupy about 2 sec., which result agrees well with the observations made upon *Branchiomma* (Nicol, 1948c). Budington (1902) has reported closely similar fatiguing effects in the longitudinal muscles of the earthworm after repetitive stimulation.

**Effects of repetitive stimulation on contractions of the longitudinal muscles**

In different groups of invertebrates there is considerable variation in the responses of muscles to repetitive stimulation. Characteristically each muscle fibre, when effectively excited, responds by an all-or-nothing contraction. In vertebrate striated muscle a single nervous impulse activates all the muscle fibres in that neuro-motor unit. A graded response results from the activity of an increasing number of neuro-muscular units. Comparable neuro-muscular studies of many invertebrate groups are still to seek. Thus Prosser & Young (1937), in a physiological investigation of the
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stellar nerves supplying the circular muscles in the mantle of the squid, found that there is normally no facilitation at the junction between the endings of the giant fibres and the muscles. Increased frequency of stimulation produces no increase in tension. They concluded that all the circular muscle fibres are activated by each nervous impulse reaching them. Some crustacean striated muscles are noteworthy, on the other hand, in that a single nervous impulse often produces no response. A series of impulses, however, causes contraction, and increase in frequency of stimulation often increases the mechanical tension developed by the muscle (review of extensive literature in Scheer, 1948, and Welsh and Schallek, 1946). Neuro-muscular facilitation has also been reported in coelenterates (pertinent papers reviewed by Scheer, 1948, and Prosser, 1946). In Calliactis, for example, a single stimulus is inadequate; repetitive stimulation initiates contraction and with each successive stimulus the contraction increases (Pantin, 1935). Against this background it is of some interest to discover the character of the mechanism that occurs in the giant fibre system of polychaetes, as exemplified by Branchiomma. The simplicity of the giant axon-longitudinal muscle organization, and knowledge of the histological picture, permit an investigation in this species.

A typical experiment with isometric contractions is shown in Fig. 6. Contractions are represented by downward deflexions of the lever in these records, which are shown in the order obtained. At a stimulation frequency of 34 per min. there is complete relaxation between contractions in this experiment. At a stimulation frequency of 64 per min. complete relaxation between separate contractions occurs for the first two stimuli, after which some fusion takes place as the muscle fatigues and relaxation time increases. Fusion becomes more complete at higher frequencies (above 2 per sec.). There is no indication of increase in tension in these records with increase in frequency of stimulation; in fact, as stimulation is prolonged, tension soon falls to zero as fatigue sets in.

A few records taken with a semi-isometric lever show some increase in tension as the frequency of stimulation is increased in the range of partial relaxation. Examples are shown in Fig. 7. Records a–e are tracings taken in that order from one specimen;

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Fig. 6. Isometric contractions. Time scales, a, b, c, 1 per sec.; d, 5 per sec. Stimulation frequencies: a, 34 per min.; b, 64 per min.; c, 116 per min.; d, 13 per sec.
An isolated record from another specimen. These results are ascribed to viscosity changes in the muscle with progress of fatigue. Budington (1902) similarly found an increase in the height of isotonic twitches in earthworm muscle under rapid stimulation. This increase in contraction paralleled, and resulted from, fatigue and prolongation of relaxation periods.

Fig. 7. Semi-isometric contractions. Time scales, a, b, c, d, e, 1 per sec.; f, 5 per sec. Stimulation frequencies: a, 36 per min.; b, 82 per min.; c, 164 per min.; d, 164 per min.; e, 12 per min.; f, 13 per sec.

The effect of increased tension on muscular elongation

Branchiomma is noteworthy in the amount of elongation it can undergo. When the entire animal is subject to stretching by gradual weighting, increase in length amounts to about 100% before the tissue ruptures (increases from an initial length of 12–26 cm. are representative figures for a mature animal). The records show certain peculiarities (Fig. 8). Initially, in all records, there are small increases in length as equal weights are added. Then at some period there are one or two steps of great increase in length, followed by a period of greatly reduced increments with increased loading. Deviation from true elasticity is shown by the partial return to original length (Fig. 8b). A strip of body wall, consisting predominantly of longi-
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The results of these experiments show that the neuro-muscular system comprising giant axons and longitudinal muscles functions in an all-or-nothing manner. A single excitable stimulus to the axons sets up a maximal muscular response, and further increments in frequency are ineffective in increasing tension when the muscle is not markedly fatigued. The histological arrangement of the giant axons indicates that an impulse, originating anywhere in either fibre, will proceed throughout the length of the body, spread out into the motor branches, and excite all the longitudinal muscles. The only synapse in the efferent side of this system lies at the neuro-muscular junction. Presumably some mechanism exists for the two giant axons to discharge synchronously, but their relationship to one another requires further study.

The structure and functioning of this system permit an interpretation in terms of the habits of the animal. The giant axons are involved in a withdrawal reflex in which the animal suddenly contracts and jerks back into its tube. Only one synergic contraction is involved normally in this response, which is maximal; a variable period of at least several minutes elapses before the animal emerges again and can be stimulated to contraction once more. Similar quick contractions attendant upon giant axon stimulation have been noted in Myxicola infundibulum, in which only one giant fibre is involved. Here it was found that increasing strength of stimulation, above threshold, is ineffective in increasing the extent of contraction, and contractions are all-or-nothing in nature. The systems in Branchiomma and Myxicola are suitable for sudden and rapid movements; apart from overcoming the inertia of the animal and building up hydrostatic pressure in the tube, little tension need be developed. The infrequency of stimulation in the normal life of the animal excludes the muscle fatigue observable under experimental conditions, and this contingency is doubly ensured by rapid adaptation in sensory pathways (Nicol, 1950).

An analogous situation has been investigated in the giant axon system of the squid (Young, 1937; Prosser & Young, 1937) in which a maximal response produced by single giant axon impulses serves to produce a sudden synergic contraction of the mantle and expulsion of a water jet in an all-or-nothing manner.

Obviously the functioning of the giant axons of Branchiomma is correlated with other neuro-muscular systems in the animal, but the exact relations still await exploration. It is probable that segmental connexions exist between the giant axons and motoneurones of the circular muscles, thereby affecting circular muscle tone during longitudinal muscle contraction. Other connexions possibly exist with...
nervous circuits serving septal and parapodial muscles in order to effect proper co-ordination of movement. Finally, some evidence is available that longitudinal muscle tone itself may be augmented following giant axon stimulation. A suggested explanation is that the giant axons secondarily discharge into segmentally arranged motoneurones that control localized responses.

The longitudinal muscles of Branchiomma, like those of other polychaetes, have striking features which distinguish them histologically from those of higher animals. In their absence of striation they can be classified as smooth muscle but they are more akin to striated muscle in their contractile properties. Physiological differences between muscles are probably quantitative, however, and these muscles of Branchiomma functionally occupy an intermediate position between the two extremes. It may be noted that in Branchiomma (and in Myxicola), synergic contraction of the animal stops at the level where the giant axons and nerve cord are cut. No evidence is thereby afforded for a syncytial muscle structure, in which a muscular excitation passes uninterrupted throughout the tissue as in vertebrate cardiac muscle. However, this is not to gainsay that whereas the sarcoplasm may be syncytial in nature the contractile filaments may still be discrete. Moreover, the transmission of a muscle potential has still to be demonstrated before this objection can be considered valid. It is worthy of note, in this connexion, that nervous rather than muscular transmission has been postulated for the intramuscular spread of excitation in insects (Roeder & Weiant, 1950). Further studies of neuromuscular physiology in polychaetes are obviously required.

SUMMARY

Synergic contractions following giant axon stimulation in Branchiomma vesiculosum have been investigated by the graphic method of recording. Isotonic and isometric levers were used, and electrical stimuli from condenser discharges were applied to the surface of the animal.

Single muscle twitches occur at stimulation frequencies up to 2 per sec., above which clonus, and finally tetanus result. At high rates of stimulation fatigue rapidly sets in; this fatigue is reversible.

Data for the time course of contractions are presented. Maximal tension develops about 255 msec. after the beginning of contraction, and relaxation occupies about 1.8 sec.

Maximal tension developed isometrically under stimulation at different frequencies (12 per min. to 13 per sec.) was measured. Maximal tension is developed initially, and there is no evidence for facilitation.

Extension of the animal and of strips of the body wall under tension are described.

The results are discussed in terms of the habits of the animal, and compared with similar studies of other invertebrates.
REFERENCES


